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SOME NEW PINE POLLINATION TECHNIQUES

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The authors are cooperating in experimental breeding of southern pines on the A. J. Hodges Industries Experimental Area near Many, in Sabine Parish, Louisiana. They began controlled pollination in 1954 with the techniques described by Cumming and Righter (1) 1/, except that, in common with several other investigators, they substituted synthetic sausage casings for cloth-and-plastic pollination bags. Their work at Many during the spring of 1954 and discussions with fellow workers elsewhere have brought to light new information and further modifications of technique which it seems desirable to share promptly with other agencies and individuals interested in forest tree improvement.

Stages of Development of Female Strobili

Figures 1-3 are offered as guides--more detailed than those in previous publications (1, 2)--to the critical stages at which female "flowers" (strobili) must be protected by bags, or at which pollen must be applied. The stages are designated according to the system of Cumming and Righter (1), who describe them as follows:

(1) Buds small, (2) buds large, (3) buds opening, (4) flowers partly open, (5) flowers maximum, (6) flowers closed, (7) cones enlarging. They add that stages 1-3 are determined by the size or condition of the bud and stages 4-7 by the position of the cone scale relative to the axis of the conelet. They describe the cone scales (not the spines) at stage 5, the optimum for pollination, as being roughly at right angles to the axis. They describe the cone scale at stage 6 as so

1/ Underscored numbers in parentheses refer to Literature Cited, page 13.

Right, stage 2-3, bud large to bud opening; *left*, stage 3, bud opening.

Stage 3-4, bud opening to flower partly open.

Stage 4, flower partly open.

Stage 5, "flower maximum" (optimum stage for pollination).

Stage 5-6, flower closing and becoming non-receptive to pollen.

Figure 1.--Developmental stages of female strobili of longleaf pine, *Pinus palustris*, according to the designations of Cumming and Righter (1).

tightly closed that pollination is no longer possible. The stages I, II, III, and IV of the earlier article by Snow and co-workers (2) correspond closely to stages 2, 3, 5, and 6 of Cumming and Righter.

In the 1954 breeding at Many, on three species of southern pine and on a natural hybrid between two of the three, visible openings between scales were found superior to scale angle as evidence that flowers were in stage 5. Stage 6 was more clearly marked by the closing of these openings and by an obvious thickening of the tips of the cone scales, which made them look like little pillows (fig. 3, *left*), than by scale angle. In stage 3, the spines of the cone scales were more or less rudimentary, depending on species; in stage 4, they were fully formed in all species, but the openings between scales that were characteristic of stage 5 had not yet appeared. For easiest recognition, especially in the smaller-flowered species, these details of development require a 12-power hand lens, but in practice, subject to occasional verification with the lens, most of them can be recognized with the naked eye.

Plainly discernible changes in color often accompany the changes in size and in scale position and thickness shown in figures 1-3. These color changes differ with species, and to some extent with trees within species. As a rule, female strobili of the southern pines become either darker, or more distinctly tinged with green,

Figure 2.--Female strobili of longleaf pine in stage 5, optimum for pollination. Flower at left intact; note that the edges of the scales, below the spines, are thin and sharp, and that wide spaces between the scales offer easy access to pollen. Flower at right bisected longitudinally to show more clearly the wide spaces between scales.

Figure 3.--Female strobili of longleaf pine in stage 6, closed and no longer receptive to pollen. Flowers have increased in diameter since stage 5. Note in intact flower to left that ends of scales have thickened so that they press closely together, with no spaces for pollen to enter; in longitudinally bisected flower to right, that gaping openings shown between scales in figure 2 have disappeared.

as they develop. Longleaf strobili, for example, usually pass from pale pink at stage 3 through brighter pink at stage 4 and bright rose at stage 5 to reddish purple at stage 6 and dark purple at stage 7.

Rates of female flower development vary greatly with species, individuals within species, season, protection or non-protection by bags, application or non-application of pollen, and probably other influences. To combine sure protection against contamination by wind-borne pollen and assurance of normal development after pollination, Cumming and Righter advocate bagging at not later than stage 2, and removal of bags after stage 7 is reached. Snow and co-workers considered bagging of slash pine in northern Florida satisfactory well into the equivalent of stage 3. On the A. J. Hodges Industries Experimental Area in 1954, flowers were bagged through stage 3 in crosses designed to produce bulk populations of hybrids from which seedlings suspected of being contaminants could be culled. For more rigorous experiments, however, an attempt was made to bag no flowers beyond stage 2 or very early stage 3.

The consensus of many workers seems to be that pollination is most successful at stage 5 and futile thereafter, but that considerable seed usually will result from pollination between stages 4 and 5 and usually some from pollination at stage 4.

Synthetic Sausage-Casing Bags

All bagging on the Hodges Experimental Area in 1954 was done with "3-1/4-inch diameter, clear, high-stretch, regular weight" moisture-permeable synthetic sausage-casing. The particular lot received came in approximately 23-inch lengths. Casings were cut into 11-1/2-inch lengths for use on longleaf, loblolly, and Sonderegger pines; lengths of 9 or 8 inches (before folding the ends to close them) proved better for all but the topmost, very vigorous twigs of shortleaf. The "3-1/4-inch diameter" (which refers to the dimensions of the sausages made from such casings; the flattened tube of the fresh, dry casing was about 4-7/8 inches across) was satisfactory for all species, but on the fastest-growing twigs of loblolly and Sonderegger pines 30-inch casings cut into 15-inch lengths would have been better than the 11-1/2-inch lengths.

Fresh casings were soft and flexible, and were easily cut, folded, stapled, and attached to the twigs. They soon stiffened under exposure to sun and air. Casings that had been stored at room temperature for a year were stiff, brittle, and much less easily folded and attached than fresh casings.

One end of each casing was stapled shut in the office before installation. The quadruple reverse fold shown in figure 4, A-C, was adopted after another worker 2/ had demonstrated that the over-and-over fold shown in figure 4D was not pollen-tight at the corners. A further refinement 3/ consists of inserting cross-wise staples (fig. 5) to keep the bag fully expanded and away from the flowers after installation.

Prior to installation of the bags, the twigs were freed of needles and wrapped with cheap non-absorbent cotton, essentially as described by Cumming and Righter (1). Ordinary pipe cleaners 4/ proved more

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2/ Bruce Zobel, Texas Forest Service, College Station, Texas.

3/ Shown to the authors by Robert G. Hitt, Department of Genetics, University of Wisconsin.

4/ Called to the authors' attention by Harold J. Derr, Southern Forest Experiment Station, Alexandria, Louisiana.

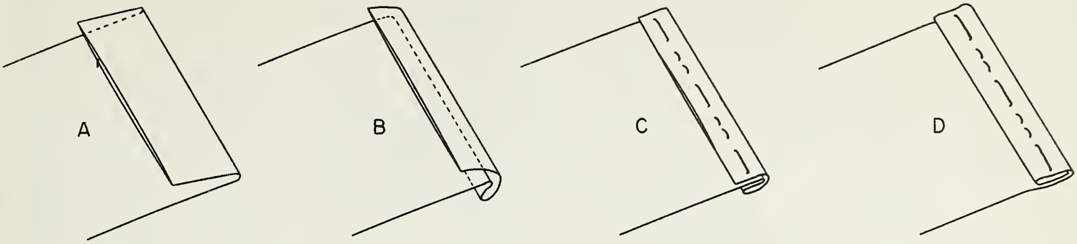

Figure 4. --A, B, C, Three stages in making and stapling pollen-tight, quadruple reverse fold in end of synthetic sausage-casing pollination bag. D, An over-and-over fold in the end of sausage casing is not pollen-tight.

satisfactory for attaching bags to twigs than did the paper-covered wires that are sold by nursery supply houses for fastening plants to stakes.

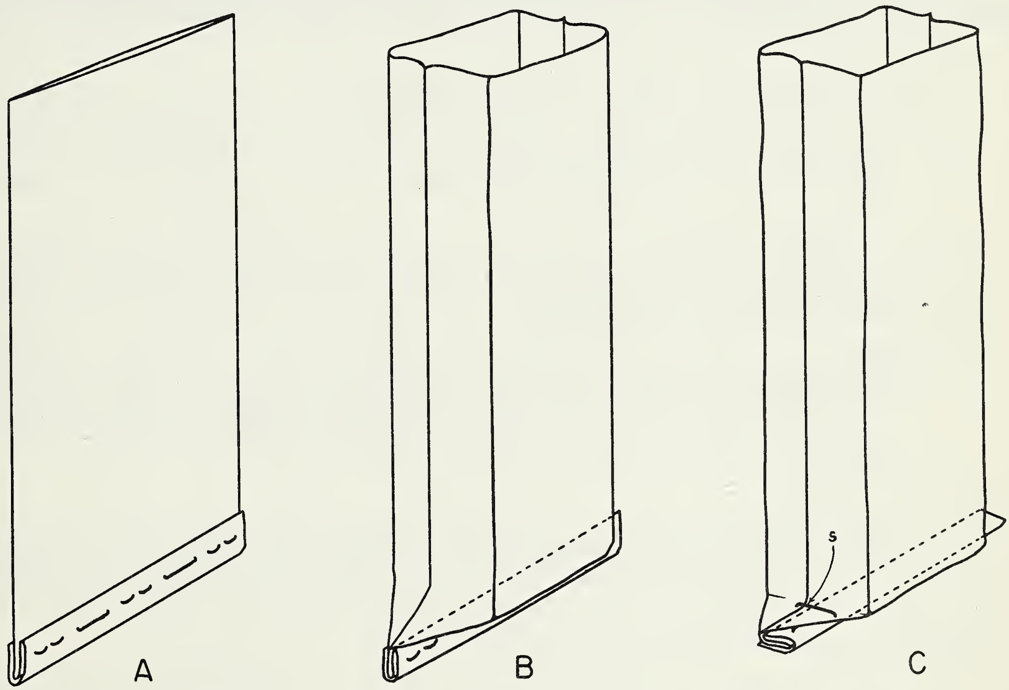

Figure 5. --Synthetic sausage-casing bag. A, With one end folded and stapled but still unexpanded. B, Expanded to maximum rectangular cross-section by blowing into it and shaping it with the fingers. C, Fixed permanently in position B by folding end seam into plane at right angles to length of bag and fastening bottom corners of expanded bag to seam with one transverse staple (s) in each side. Bags are prepared as in A in the laboratory; steps B and C are carried out in the tree, just before attaching bag to twig.

Disposable Pollen Extractor

To meet immediate needs, some attention was focused on development of a pollen extractor less elaborate and expensive than the large-scale apparatus described by Cumming and Righter (1). Laboratory space was not yet available for the special room, sink, sterilizer, and large funnels and bags described by those authors or for the blower and manifold they subsequently developed to aerate their extractor. There was need for separate extraction of numerous individual lots of pollen smaller than those for which the large funnels and bags were especially designed. Lastly, under the climatic conditions of the South, difficulty had been encountered with molding of catkins and pollen in quantities as great as those for which Cumming and Righter's equipment was best fitted.

The unit extractor developed at Many in 1954 consists of the following (fig. 6):

1. A kraft paper bag of 8-pound capacity.

2. A screen-wire cage, 5 inches deep and of a diameter to permit easy insertion into the paper bag.

3. A plastic funnel 2-1/2 inches from rim to outlet, and with a rim 2-7/8 inches in diameter; the opening of this funnel is covered with fine voile or batiste.

4. Plastic adhesive tape (1 inch wide) to shape the bag, surgical adhesive tape (1/2-inch wide) to fasten funnel in bag, string to suspend the extractor, and absorbent cotton to plug outlet of funnel.

Both the small plastic funnels (usually obtainable for 5¢ apiece at dime stores) and the 8-pound kraft bags vary somewhat in dimensions from source to source. With due allowances for such variations, construction of the extractor is as follows:

a. For the cage to hold the catkins, cut a 5- by 18-inch rectangle of 16-mesh galvanized screen wire and shape it into a cylinder 5 inches deep and 5-2/5 inches in diameter by overlapping the ends 1 inch and stapling them together. Cut two disks of screen wire each 6-2/5 inches in diameter. Around the edge of each make cuts about 1 inch apart and extending 1/2 inch toward the center of the disk. Bend the tabs so

Figure 6.--Component parts of unit pollen extractor: 8-pound capacity kraft paper bag (*left*, uncut; *right*, with end and corners trimmed for shaping to fit funnel); screen-wire cage containing catkins (top to be stapled shut before insertion in kraft bag); and 2-7/8-inch plastic funnel covered with voile.

formed up at right angles to the main disk. Insert one such disk 1/2 inch in the bottom of the cylinder and staple each tab to the side of the cylinder. Insert the other disk in the top of the cylinder and staple half-way round, leaving the other half unfastened and bent upward (fig. 6) to permit pouring catkins into the cylinder.

b. To cover funnel with voile, stretch the voile tightly over a sheet of waxed paper on a smooth table top, coat rim of funnel with quick-drying acetate cement, and press rim down on voile till cement dries; trim off the voile close to the funnel with a razor blade.

c. To shape the kraft bag, spread to maximum width and flatten out. Cut open end square and mark the mid-point. From points 5 inches below the square-cut corners of the open end, to points 1-3/8 inches on either side of the marked mid-point of the open end, draw two pencil lines. Cut off the corners of the bag along these lines (bag to right in fig. 6).

d. Place catkins in the wire cage and staple the cage shut. Place cage in the bag. Draw cut edges of bag together, and seal into position

Figure 7.--Trimmed kraft bag with screen-wire cage inside, and cut edges of bag held together with 1-inch plastic adhesive tape. Voile-covered funnel being inserted by tipping it at angle from vertical.

Figure 8.--Extractor completely assembled, with funnel turned vertically, pulled up as far as it will go into the narrowed mouth of the bag, and fastened firmly in place with two turns of 1/2-inch surgical adhesive tape outside the bag.

Figure 9.--Extractor inverted and hung up to permit catkins to dry. Note cotton plug in outlet of funnel; this is essential to prevent loss of pollen as it is shed from the catkins.

with strips of 1-inch plastic tape on both inside and outside of the bag (fig. 7); this is most easily done by passing the mouth of the bag over a smooth, slightly rounded strip of wood nailed to a table and projecting 1 foot beyond its edge.

e. Insert funnel in mouth of bag by tipping funnel as shown in figure 7. (If bag has been cut and taped correctly for size of funnel used, it will admit funnel easily in this position but will not permit it to be withdrawn when turned vertically.) Straighten funnel to vertical position, pull upward snugly into tapering mouth of bag, and fasten securely in place by two turns of 1/2-inch surgical adhesive tape, centered on rim of funnel, around the outside of the bag.

f. Plug the outlet of the funnel with absorbent cotton, tie the bottom of the bag firmly around the lower part of the cage with a double turn of soft cord, and to this cord fasten a bridle by which to hang up the extractor (fig. 8); invert and hang the extractor (fig. 9) in a warm, dry place.

At Many in 1954, best results were obtained with this extractor by placing in the cage a 1-inch layer of unwashed catkins from which pollen was just beginning to be shed. Such a charge usually yielded practically all its pollen in 24 to 48 hours.

It was demonstrated, however, that for rigorous experiments the extractor could be used with the washing technique described by Cumming and Righter (1). Catkins not quite ready to shed were caged and washed, and cage and catkins were inserted under water in an uncut 8-pound kraft paper bag, essentially as described by those authors for insertion in the cloth bag they used. The mouth of the bag was closed under water, withdrawn from the water still closed, and taped shut. All free water was shaken out of the catkins and cage and drained out through the folds closing the mouth of the bag, without exposing the catkins to the open air with its possible load of pollen. In this process, the kraft bag itself absorbed most of any water clinging to the sides of the cage.

The wet kraft bag was then removed, and replaced with a cut and fitted bag, as described under (d) above, in a pollen-free atmosphere. A pathologist's inoculation chamber or a chemical hood might be used to provide such an atmosphere. In practice, some pollen was transferred in a shower bath in which the air had been washed by running the hot shower for several minutes.

During the 1954 season, no trouble was experienced with molding of catkins or pollen in this extractor, with either unwashed or washed catkins. The only extraction failures resulted from misjudging the maturity of the catkins or the heat to which to expose them. Fully mature catkins in extractors hung in an unused greenhouse released pollen satisfactorily.

After extraction of pollen, the kraft bag, the voile on the funnel, and the cotton plug are thrown away. Cage and funnel are washed in hot, soapy water, and, as a double precaution against contamination, rinsed in isopropyl alcohol. Fresh voile is cemented onto the funnel, a new kraft bag is prepared, and the next batch of pollen extracted.

Loaded as described, the kraft-bag extractor may yield 10 to 20 cc. of pollen.

Smaller quantities of pollen can be extracted in 10- or 12-inch segments of synthethic sausage-casing of the type used for bagging female strobili. 5/

One method is to staple the bottom of a casing shut (pollen-tight), place a handful of catkins in the casing, staple a pollen-tight fold (above the catkin), and hang the casing by its top in a warm place; pollen discharged and shaken into the bottom of the casing is then withdrawn into a syringe, the needle of which is thrust through the casing 6/. A possible disadvantage of this method is lack of means of sifting the pollen, which, if it contains fragments of catkins, or the small larvae frequently found in freshly extracted pollen, may clog the needle.

An alternative method is to fill a segment of casing nearly full of catkins, staple pollen-tight seams at both ends, and hang it up to dry. When the pollen is discharged, one end of the casing is cut off and the opening fitted tightly over a funnel of suitable size 7/, through which the pollen can be shaken out into a vial. A funnel covered with voile or batiste, as previously described, frees the pollen of larvae and trash.

5/ Originally suggested by R. E. Schoenike, Southern Forest Experiment Station, Crossett, Arkansas, in 1953.

6/ Called to the authors' attention by François Mergen, Southeastern Forest Experiment Station, Lake City, Florida.

7/ Reported by Robert G. Hitt, Department of Genetics, University of Wisconsin, Madison, Wisconsin.

The moisture permeability that gives synthetic sausage casings their advantage over moisture-proof substances for bagging female strobili apparently makes them equally good containers in which to extract pollen. All three of the co-workers cited (footnotes 5, 6, 7) report good yields of pollen from casing extractors, with no molding. The casings are, moreover, inexpensive and extremely simple to prepare.

Pollinizer

For some time, glass or preferably steel and plastic hypodermic syringes of 10 cc. capacity equipped with long No. 16 veterinary needles and with special rubber bulbs instead of plungers (1) have been standard pollinating equipment.

A unit pollinizer of this type costs $3.00 to $4.00. Glass syringes are easily broken. The needles must be thrust into corks or the pinched ends of empty .22 long rifle cartridges for safe transportation up the tree. Long needles sometimes become clogged with pollen and must be cleared with a special rod or the wire of a twig tag.

In an attempt to find a cheaper and better pollinizer, 1-ounce rubber ear syringes (or, for larger capacity, bulbs of babies' enema syringes) were tried (fig. 10). These retail for 39¢ to 49¢, and, with needles at 25¢, reduced the price of the individual pollinizer to one-fourth or one-sixth of that paid for glass syringes with special bulbs instead of plungers.

The neck of the ear syringe was cut off at a point such that the orifice left formed a tight joint around the shank of the veterinary needle below the knurled portion (fig. 10). With the enema syringe, the hard-rubber nose was pulled out, and the knurled portion of the needle seated snugly in the neck of the bulb.

A 3/4-inch No. 16 veterinary needle was found preferable to longer needles. The bulb was filled with pollen before the tree was climbed. For transportation up the tree, the needle was inserted in the bulb with the point inward and held in place with 1-inch-wide plastic adhesive tape. For pollination, tape and needle were removed and the needle reinserted point outward. Discharge of pollen was best with the needle pointed horizontally or somewhat downward, depending on whether the bulb was nearly full or nearly empty. Very slight compression of the bulb injected ample pollen into the bag. The 1-ounce ear syringe carried enough pollen for 100 or more bags.

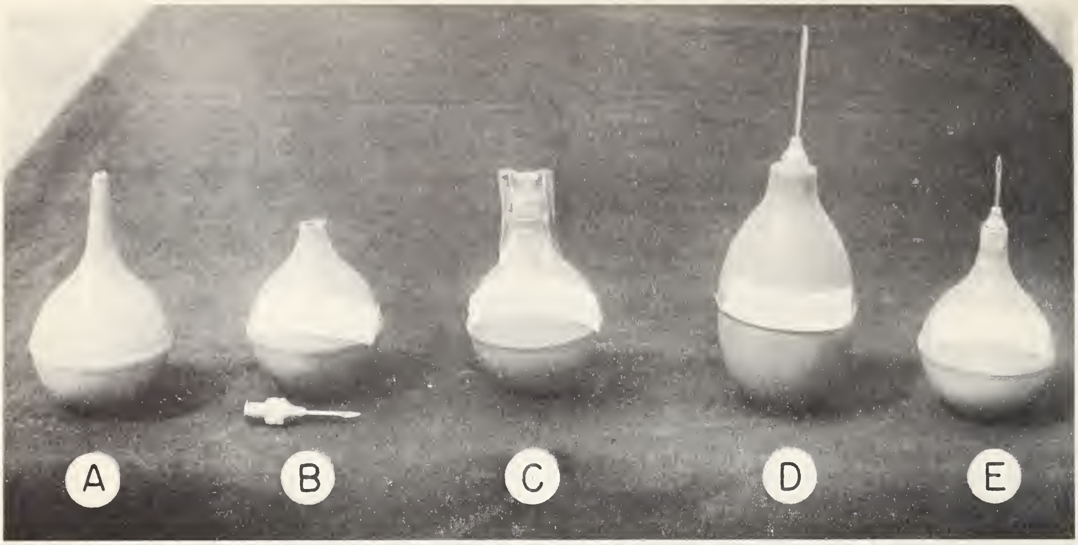

Figure 10.--A.--Unmodified 1-ounce rubber ear syringe. B.--Ear syringe with neck cut off to permit snug insertion of shank of the 3/4-inch No. 16 veterinary needle lying in front of bulb. C.--Ear syringe with needle taped in place, point inward, for carrying up tree. D.--Babies' enema bulb with (longer) No. 16 needle in place for pollen injection. E.--Ear syringe with 3/4-inch No. 16 needle in place for pollen injection. The bands of surgical adhesive tape on the bulbs are for marking, in pencil, the kinds of pollen contained.

The low cost of this pollinizer permitted use of separate units for many different lots of pollen. Any cleaning and sterilizing of pollinizers while in the tree, and usually any refilling of syringes in the tree, was therefore unnecessary. Ordinarily, no glassware of any kind had to be carried up trees. After pollinizers had been used and the needles had been turned point inward and taped into place, the bulbs could be dropped to the ground without injury or loss of pollen. Bulbs and needles were thoroughly cleaned with hot, soapy water before re-use, and further washed with isopropyl alcohol to kill any adhering grains of pollen. It was found, however, that this alcohol caused rapid deterioration of the rubber bulbs.

When pollen was carried into the field in 10-cc. vials with small necks, the close fit between shoulder of bulb and neck of vial made it easy to transfer pollen from vial to bulb (by gravity and slight suction, figure 11) or back from bulb to vial (by gravity alone) with little or no spilling and no appreciable danger of contamination, even in a high wind.

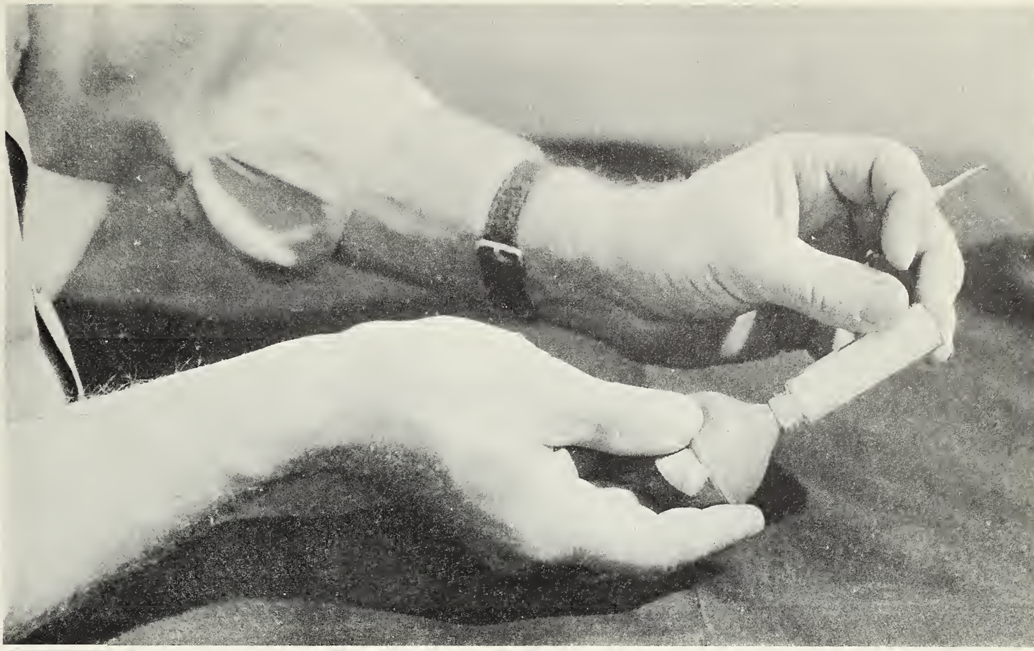

Figure 11.--Ear syringe pollinizer, with needle removed, being filled with pollen from small vial by gravity and slight suction.

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